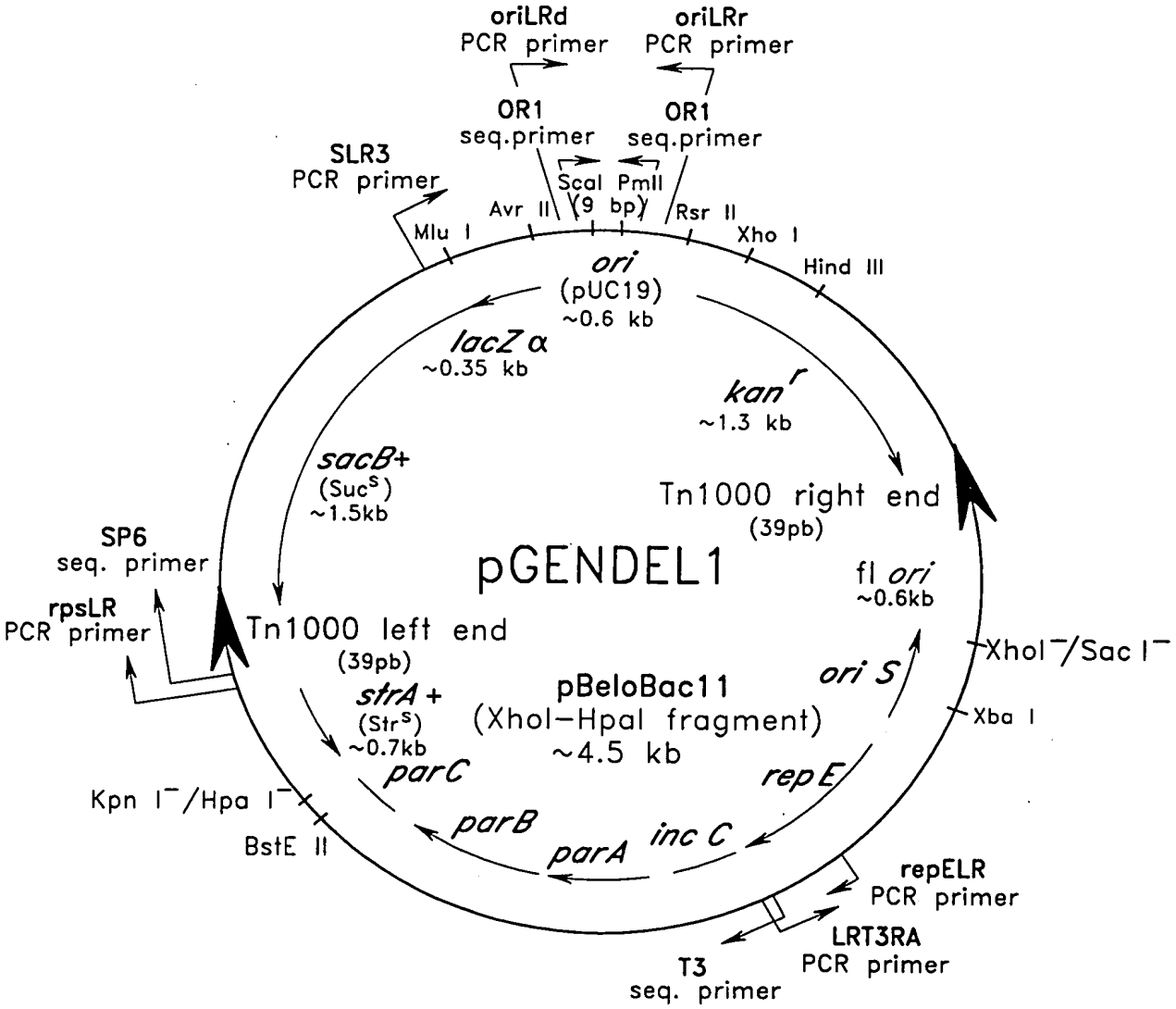
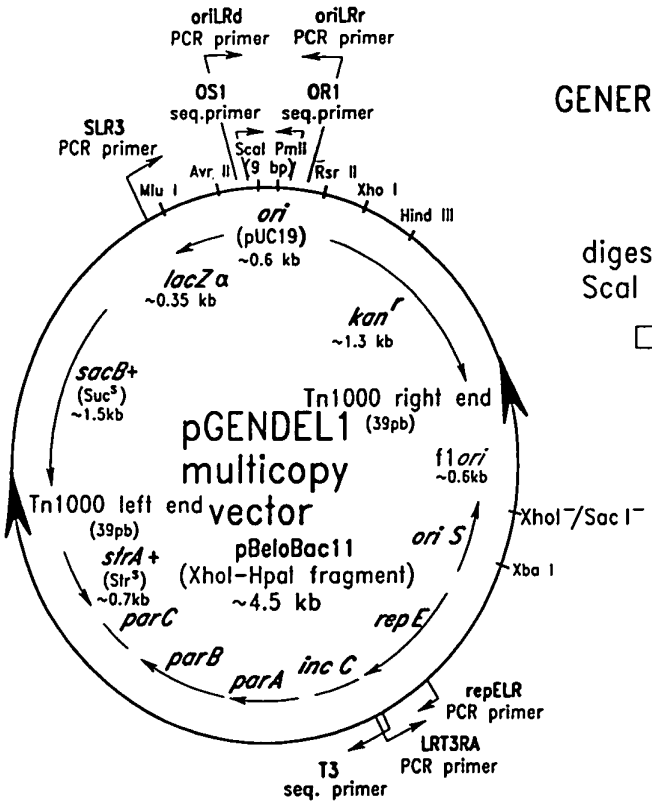

oriLRd
PCR primer
oriLRr
PCR primer
OR1
seq.primer
OR1
seq.primer
SLR3
PCR primer
ScaI PmlI
(9 bp)
Avr II
Rsr II
Mlu I
Xho I
Hind III
ori
(pUC19)
~0.6 kb
lacZ α
~0.35 kb
kan r
~1.3 kb
sacB+
(Suc S)
~1.5kb
Tn1000 right end
(39pb)
SP6
seq. primer
pGENDEL1
fl ori
~0.6kb
rpsLR
PCR primer
Tn1000 left end
(39pb)
XhoI−/Sac I−
ori S
pBeloBac11
(XhoI−HpaI fragment)
~4.5 kb
strA +
(Str S)
~0.7kb
Xba I
parC
repE
Kpn I−/Hpa I−
parB
inc C
BstE II
parA
repELR
PCR primer
LRT3RA
PCR primer
T3
seq. primer

FIG. 1

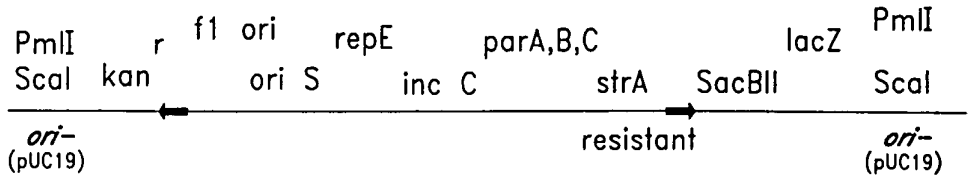
FIG.2

FIG.2A
FIG.2B
FIG.2C



GENERATION OF NESTED DELETIONS IN pGenDel VECTOR
BY TRANSPOSASE ACTION

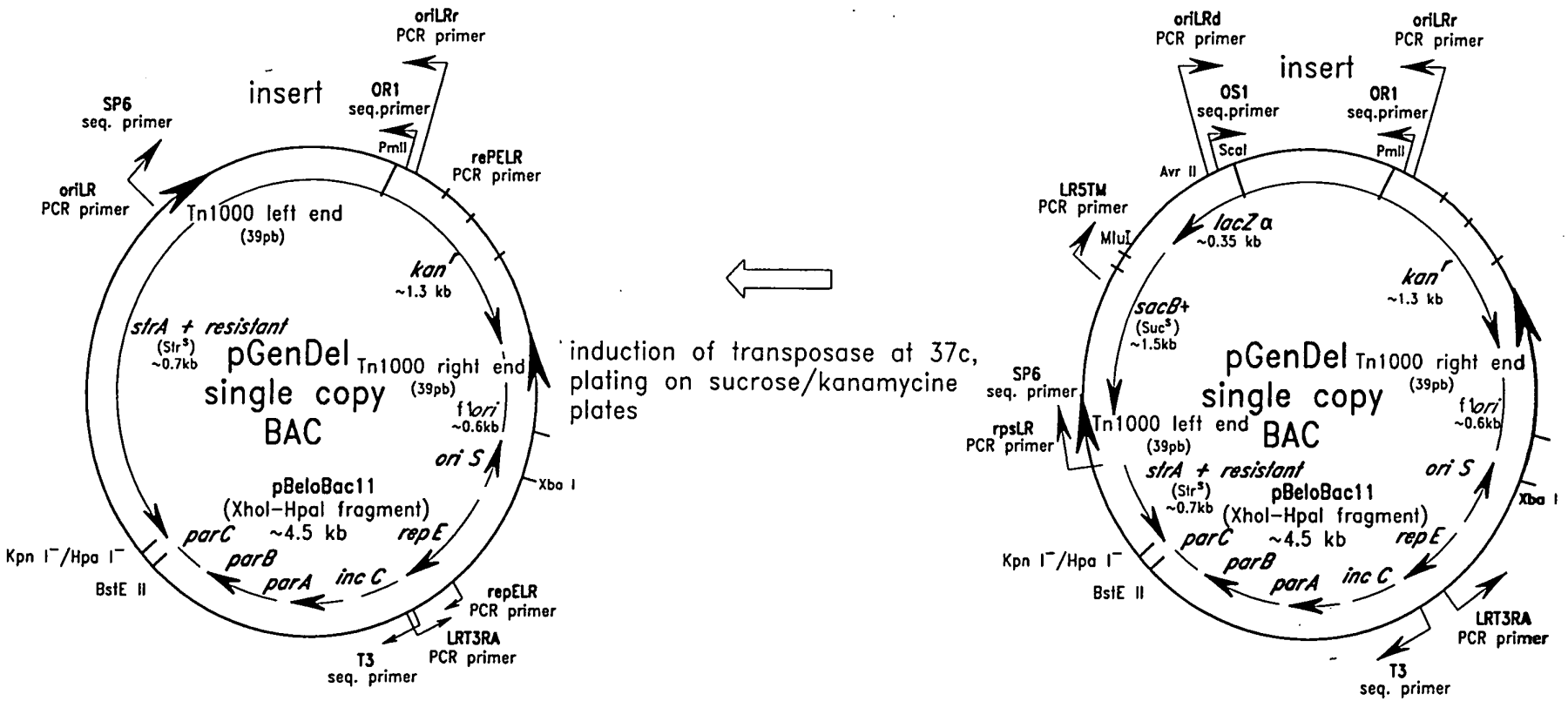
digestion with
ScaI or PmlI



ligation with blunt-ended
DNA fragment
electroporation into
cells containing transposase

kanamycine resistant, streptomycine sensitive if introduced into streptomycine resistant host cells, sucrose sensitive deeply on IPTG/Xgal plates

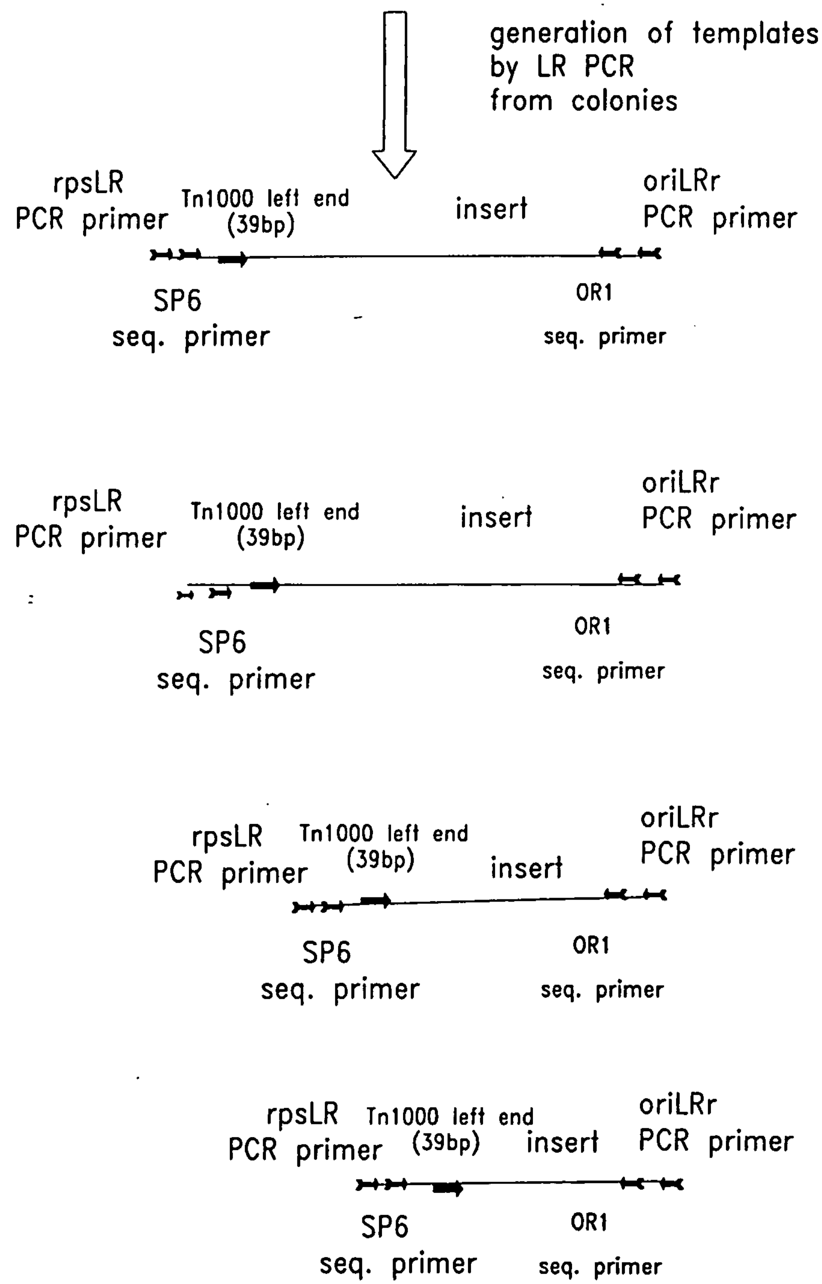
FIG.2A



kanamycine resistant, streptomycine resistant, sucrose resistant white on IPTG/Xgal plates

kanamycine resistant, streptomycine resistant if introduced into streptomycine resistant cells, sucrose sensitive faint blue on IPTG/Xgal plates

FIG.2B



1.Forced cloning of blunt ended fragments into pGenDel by contra-selection on streptomycine plus kanaycine

2. Selection of intra transposed clones by plating on sucrose/kanamycine/Xgal media

3. Generation of templates by PCR from colonies.

4. Minimal tiling path determination by sizing

FIG.2C

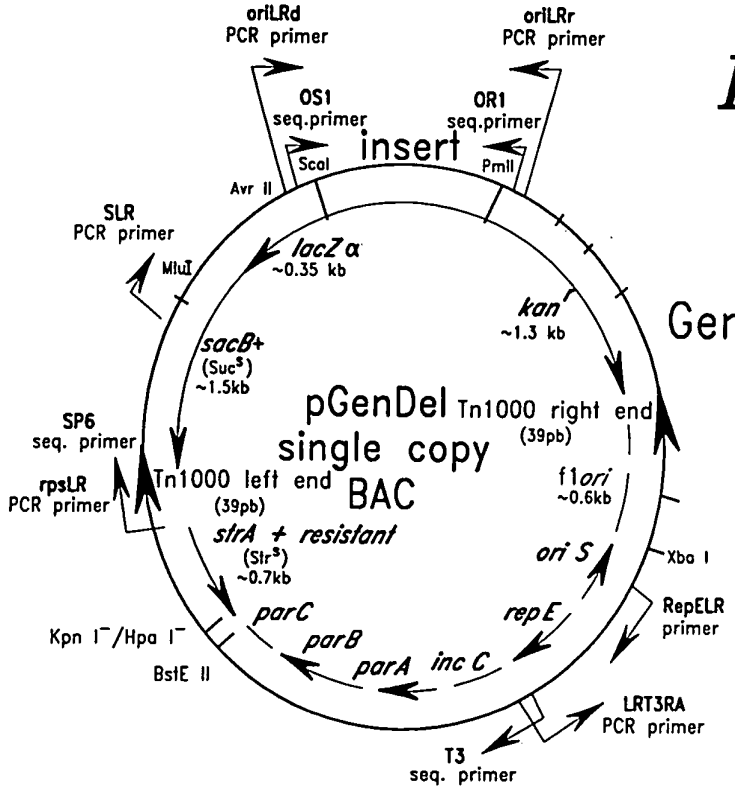


FIG. 3

FIG.3A
FIG.3B
FIG.3C

Generation of nested deletions with exoIII

kanamycine resistant, streptomycine resistant if introduced into streptomycine resistant cells, sucrose sensitive, faint blue on IPTG/Xgal plates

generation of linear substrates by LR PCR with SLR3 and LRT3RA primers from cells

SLR 3 primer →

lacZ ori− (pUC19) PmlI ScaI INSERT ScaI PmlI ori− (pUC19) f1 ori kan repE ori S

5/TpTpTpCpGpCpG ---------- CpGpGpCpTpTpT3/

3/ApApApGpCpGpC ---------- GpCpCpGpApApA5/

← LRT3RA primer

FIG. 3A

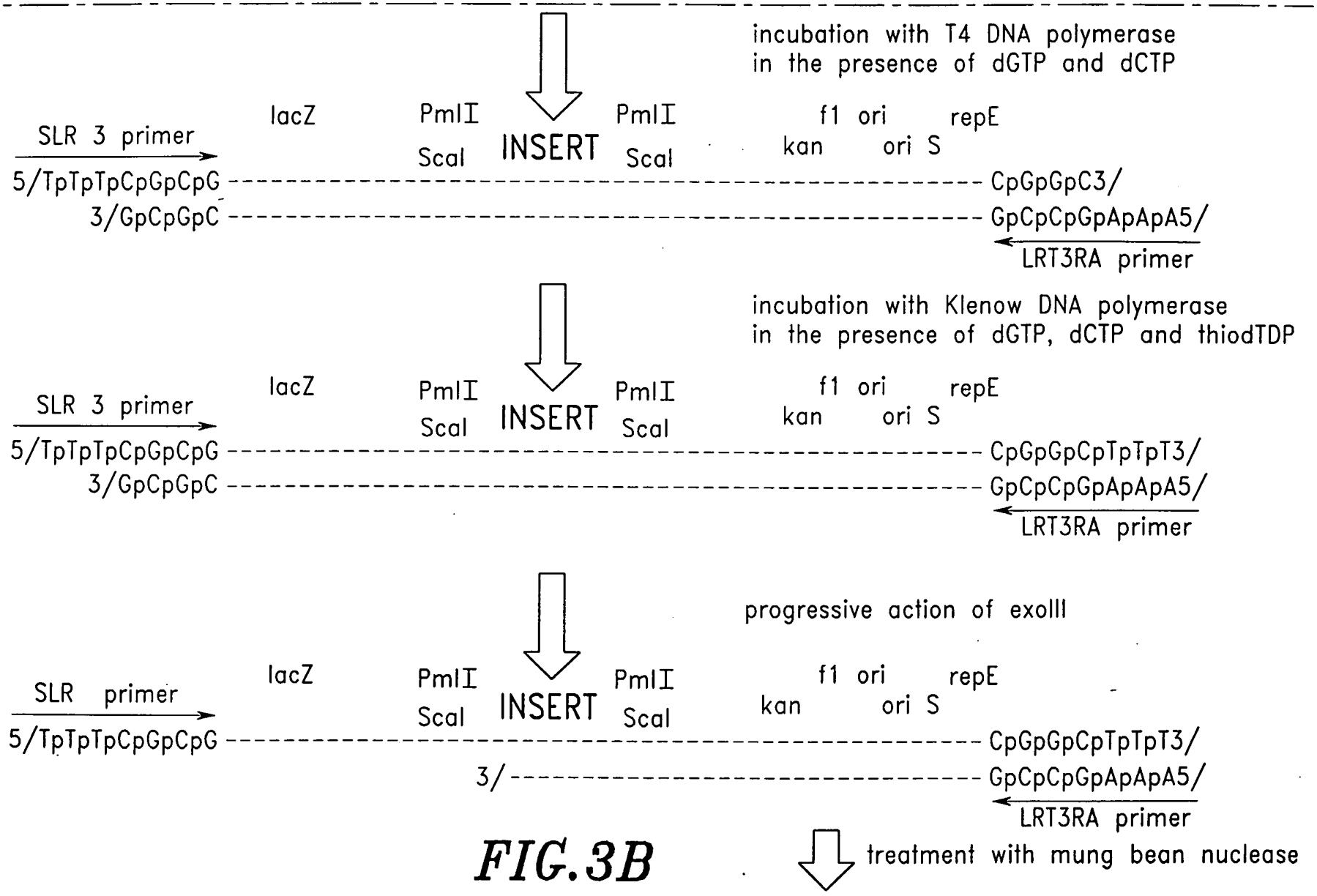
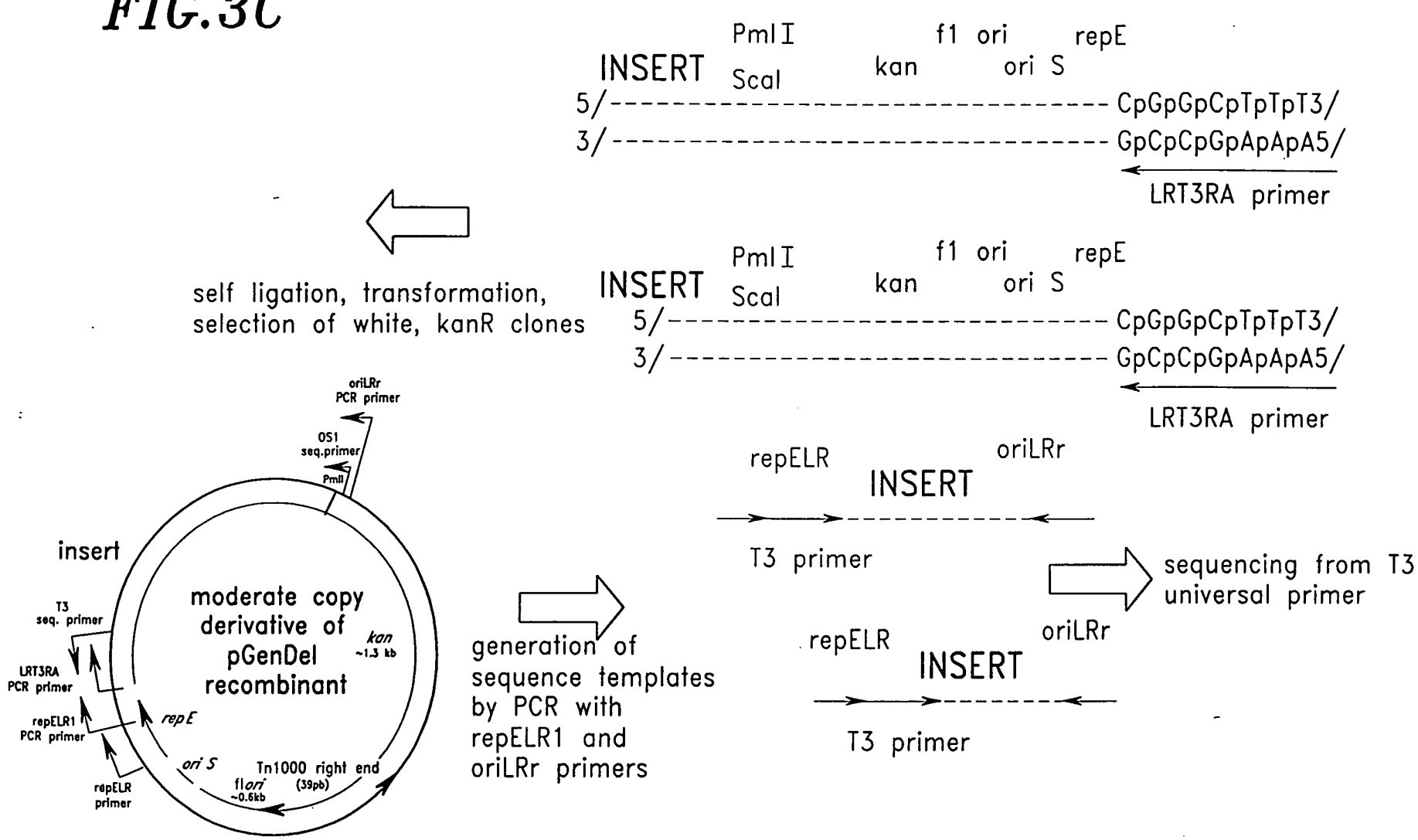

incubation with T4 DNA polymerase
in the presence of dGTP and dCTP
SLR 3 primer
lacZ
PmlI
ScaI
INSERT
PmlI
ScaI
f1 ori
repE
kan
ori S
5/TpTpTpCpGpCpG
CpGpGpC3/
3/GpCpGpC
GpCpCpGpApApA5/
LRT3RA primer
incubation with Klenow DNA polymerase
in the presence of dGTP, dCTP and thiodTDP
SLR 3 primer
lacZ
PmlI
ScaI
INSERT
PmlI
ScaI
f1 ori
repE
kan
ori S
5/TpTpTpCpGpCpG
CpGpGpCpTpTpT3/
3/GpCpGpC
GpCpCpGpApApA5/
LRT3RA primer
progressive action of exoIII
SLR primer
lacZ
PmlI
ScaI
INSERT
PmlI
ScaI
f1 ori
repE
kan
ori S
5/TpTpTpCpGpCpG
CpGpGpCpTpTpT3/
3/
GpCpCpGpApApA5/
LRT3RA primer
treatment with mung bean nuclease

FIG. 3B

FIG.3C

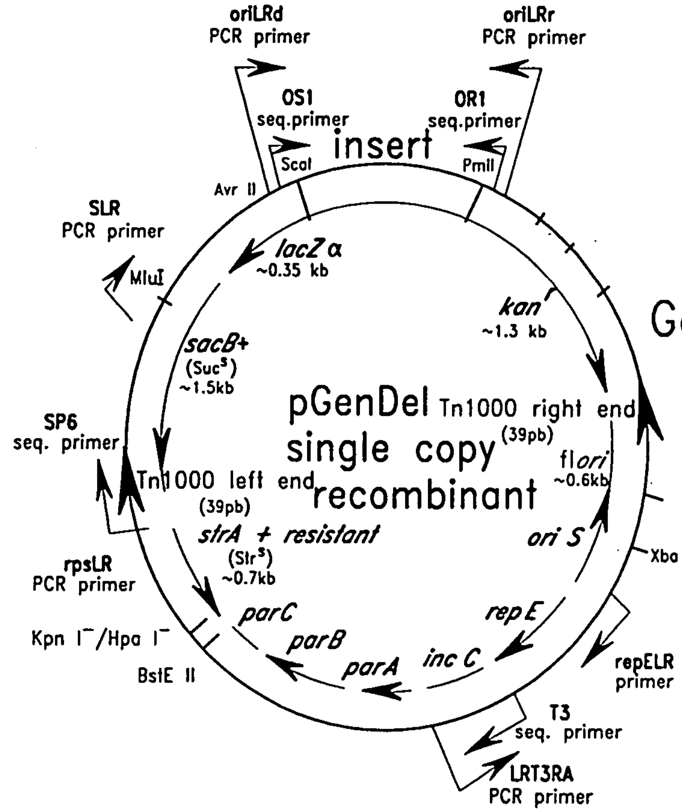


kanamycine resistant, streptomycine resistant if introduced into streptomycine resistant cells, sucrose sensitive, white on IPTG/Xgal plates

FIG. 4

FIG.4A
FIG.4B
FIG.4C

Generation of nested deletions with Mung bean nuclease



kanamycine resistant, streptomycine resistant if introduced into streptomycine resistant cells, sucrose sensitive, faint blue on IPTG/Xgal plates

generation of linear substrates by LR PCR with SLR3 and LRT3RA primers from cells

5/TpTpTpCpGpCpG -- CpGpGpCpTpTpT3/
3/ApApApGpCpGpC-- GpCpCpGpApApA5/

SLR 3 primer → lacZ ori- (pUC19) PmlI ScaI INSERT PmlI ScaI ori- (pUC19) kan f1 ori ori S repE ← LRT3RA primer

FIG. 4A

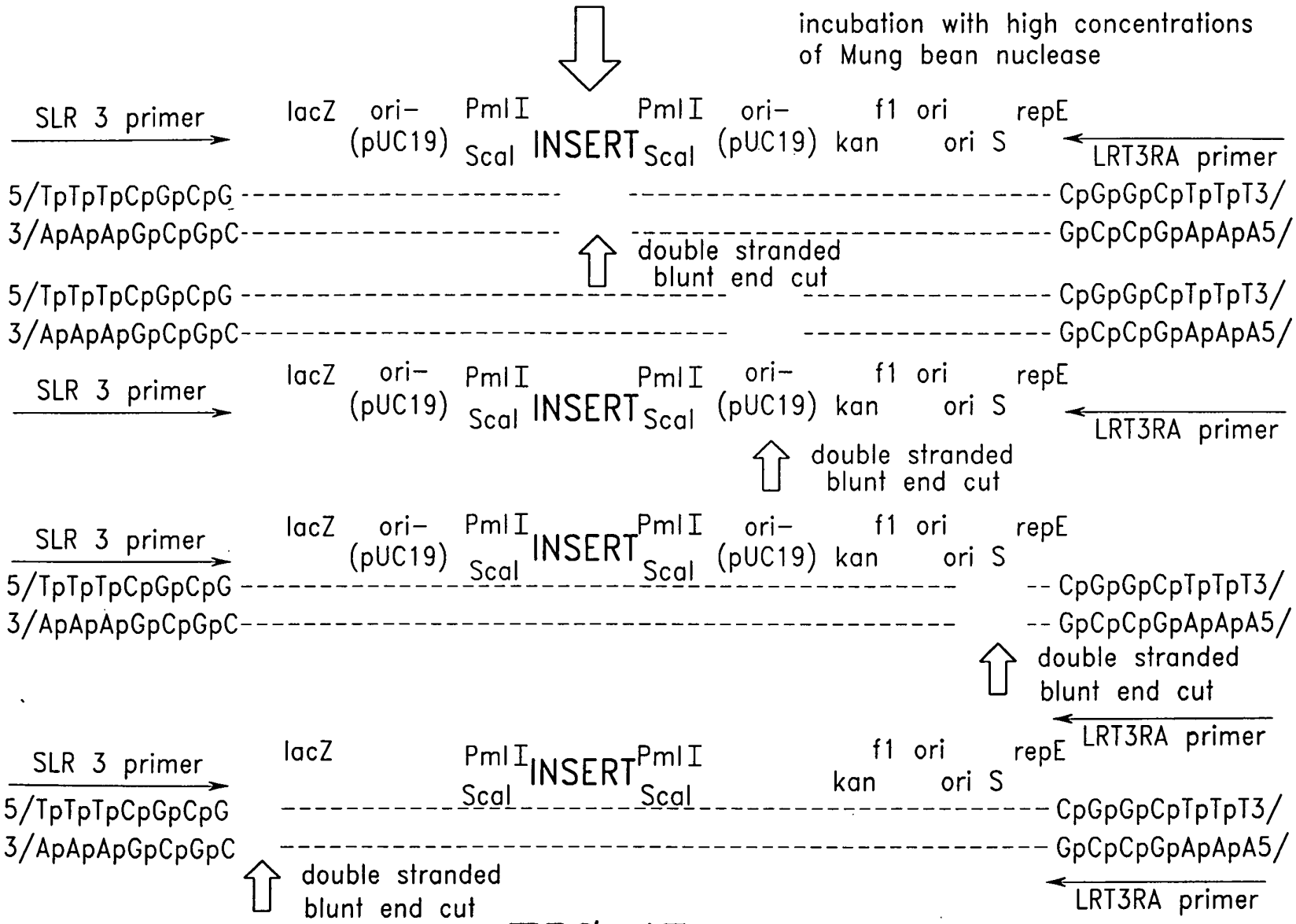

incubation with high concentrations of Mung bean nuclease
SLR 3 primer
lacZ
ori-(pUC19)
PmlI ScaI
INSERT
PmlI ScaI
ori-(pUC19)
f1 ori
kan
ori S
repE
LRT3RA primer
5/TpTpTpCpGpCpG
CpGpGpCpTpTpT3/
3/ApApApGpCpGpC
GpCpCpGpApApA5/
double stranded blunt end cut

FIG.4B

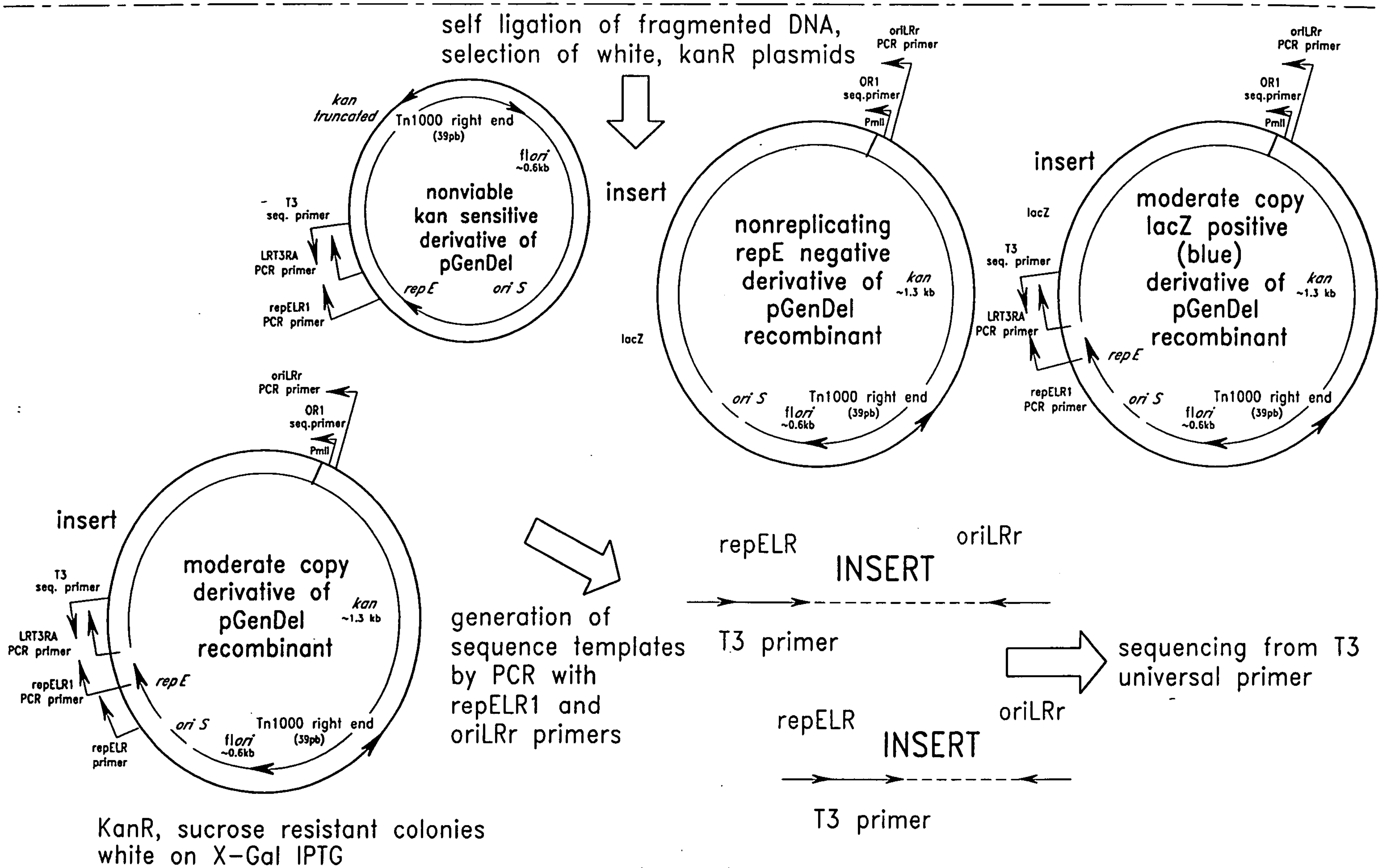
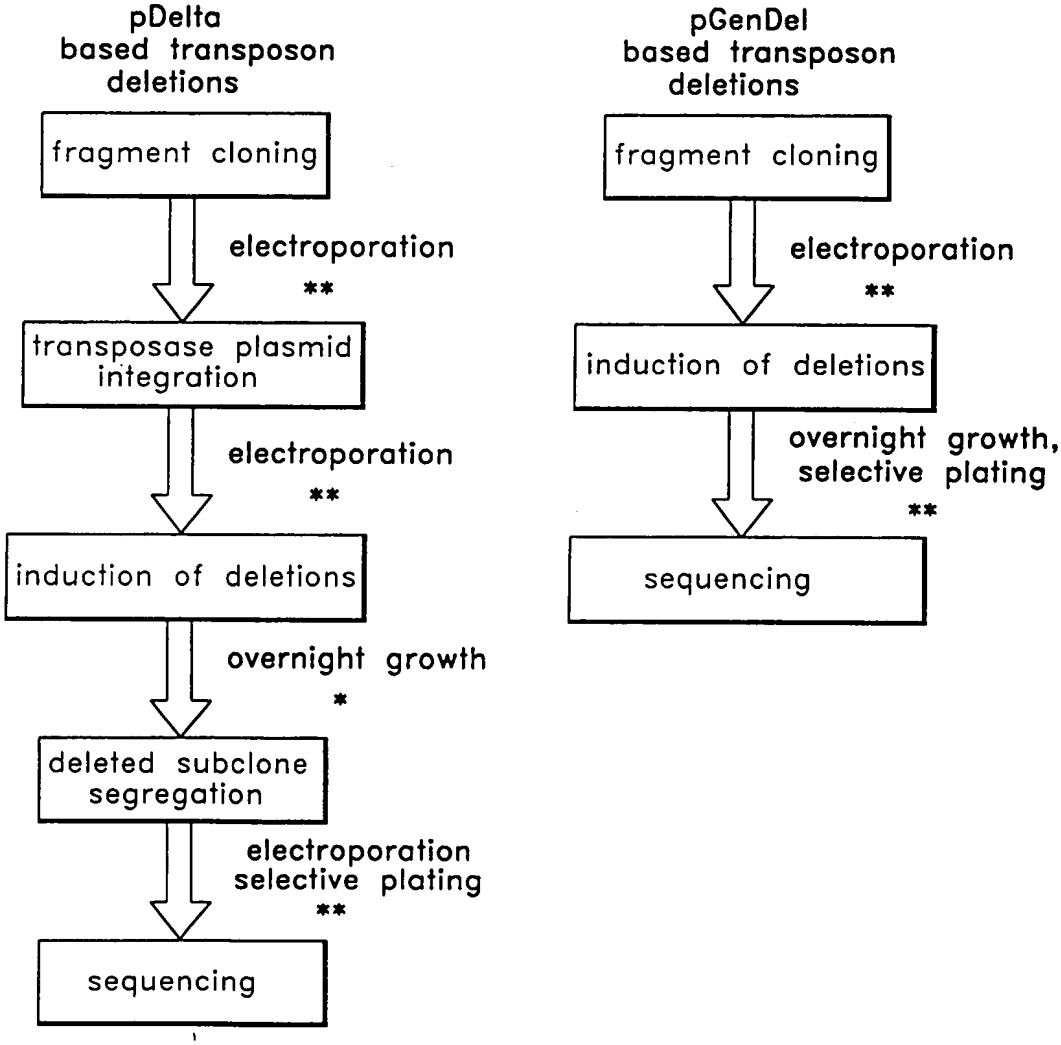

self ligation of fragmented DNA,
selection of white, kanR plasmids
kan truncated
Tn1000 right end (39pb)
flori ~0.6kb
nonviable kan sensitive derivative of pGenDel
T3 seq. primer
LRT3RA PCR primer
repELR1 PCR primer
repE
ori S
insert
lacZ
oriLRr PCR primer
OR1 seq.primer
PmlI
nonreplicating repE negative derivative of pGenDel recombinant
kan ~1.3 kb
moderate copy lacZ positive (blue) derivative of pGenDel recombinant
moderate copy derivative of pGenDel recombinant
repELR primer
KanR, sucrose resistant colonies
white on X-Gal IPTG
generation of sequence templates by PCR with repELR1 and oriLRr primers
repELR
INSERT
oriLRr
T3 primer
sequencing from T3 universal primer

FIG.4C

FIG. 5 FIG.5A FIG.5B

Comparison of different methods of nested deletion sequencing



*shown in *– easy stages
**– difficult stages
***–very difficult stages

FIG. 5A

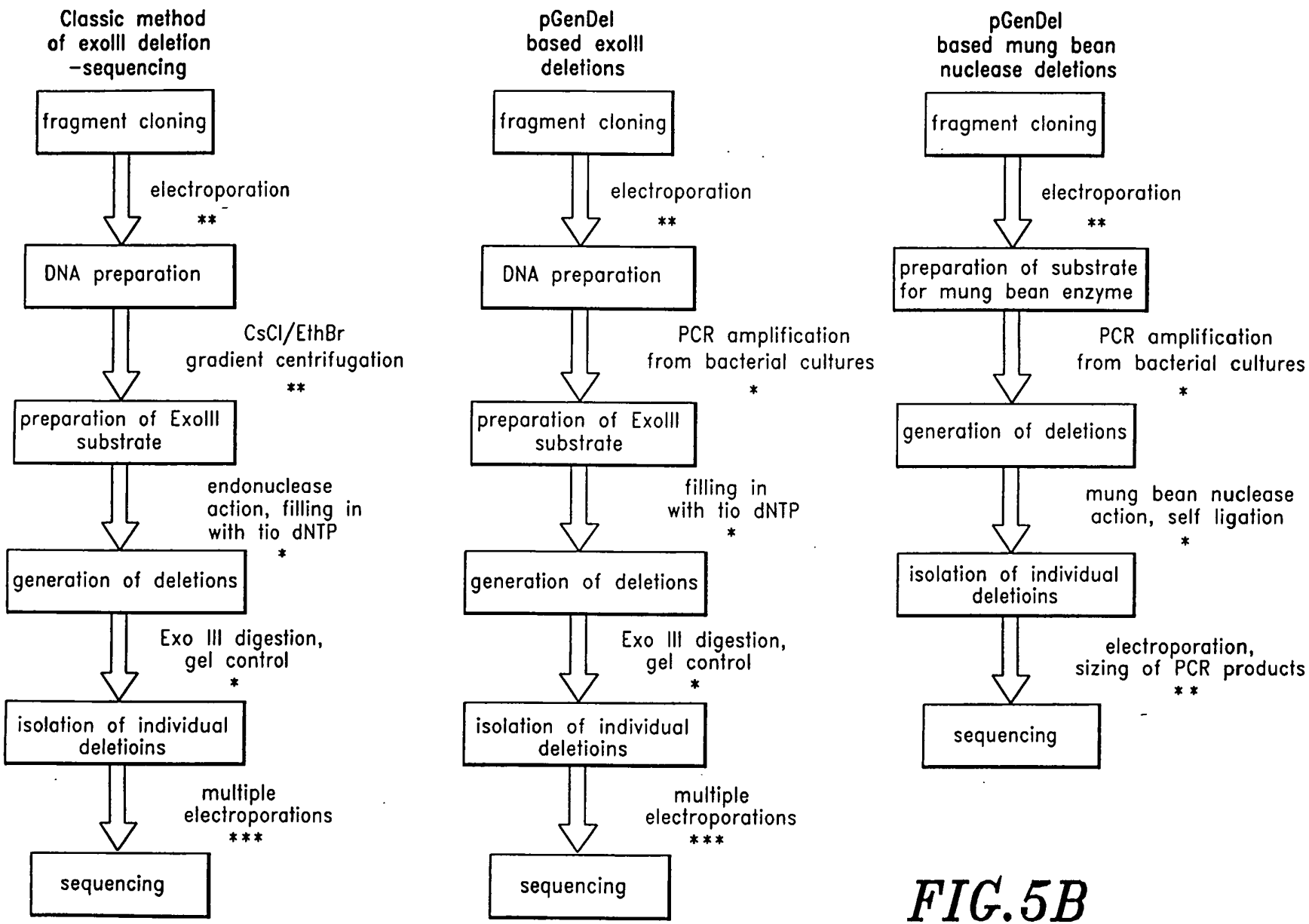


FIG.5B

FIG. 6

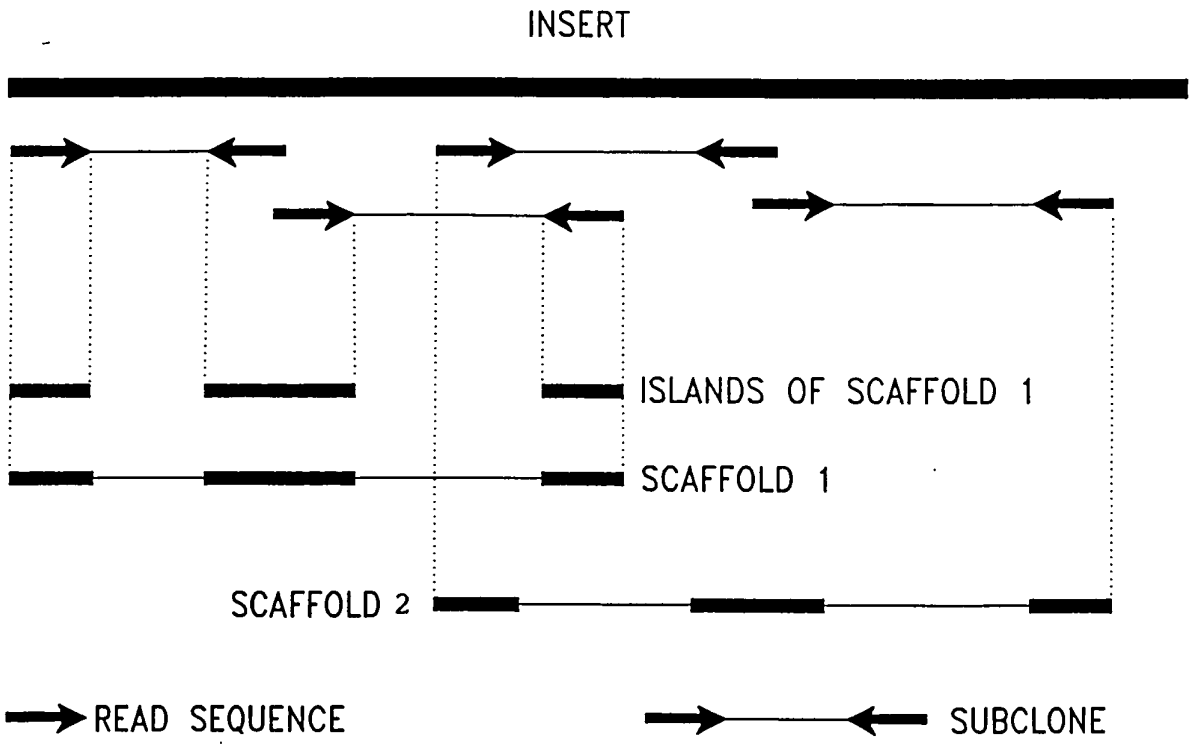


FIG. 7

MULTIPLE NUCLEATION POINT

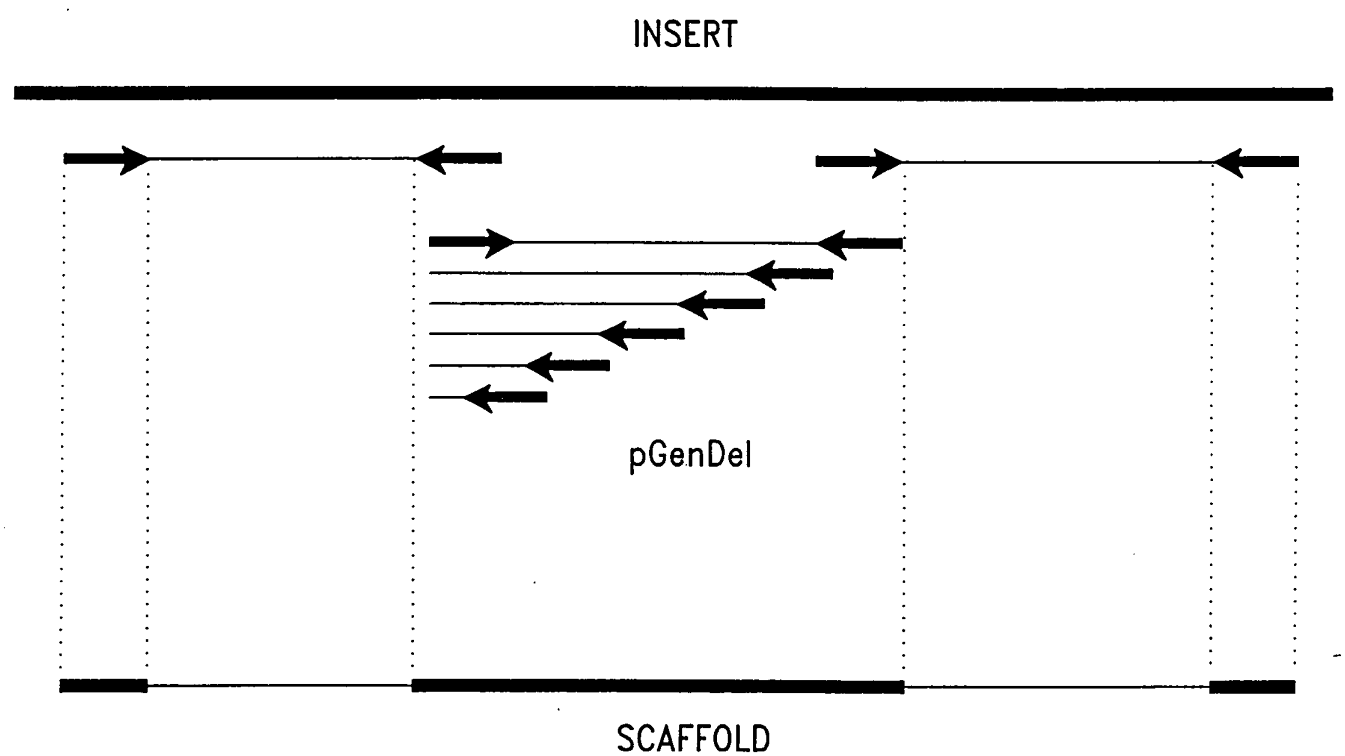


FIG.8

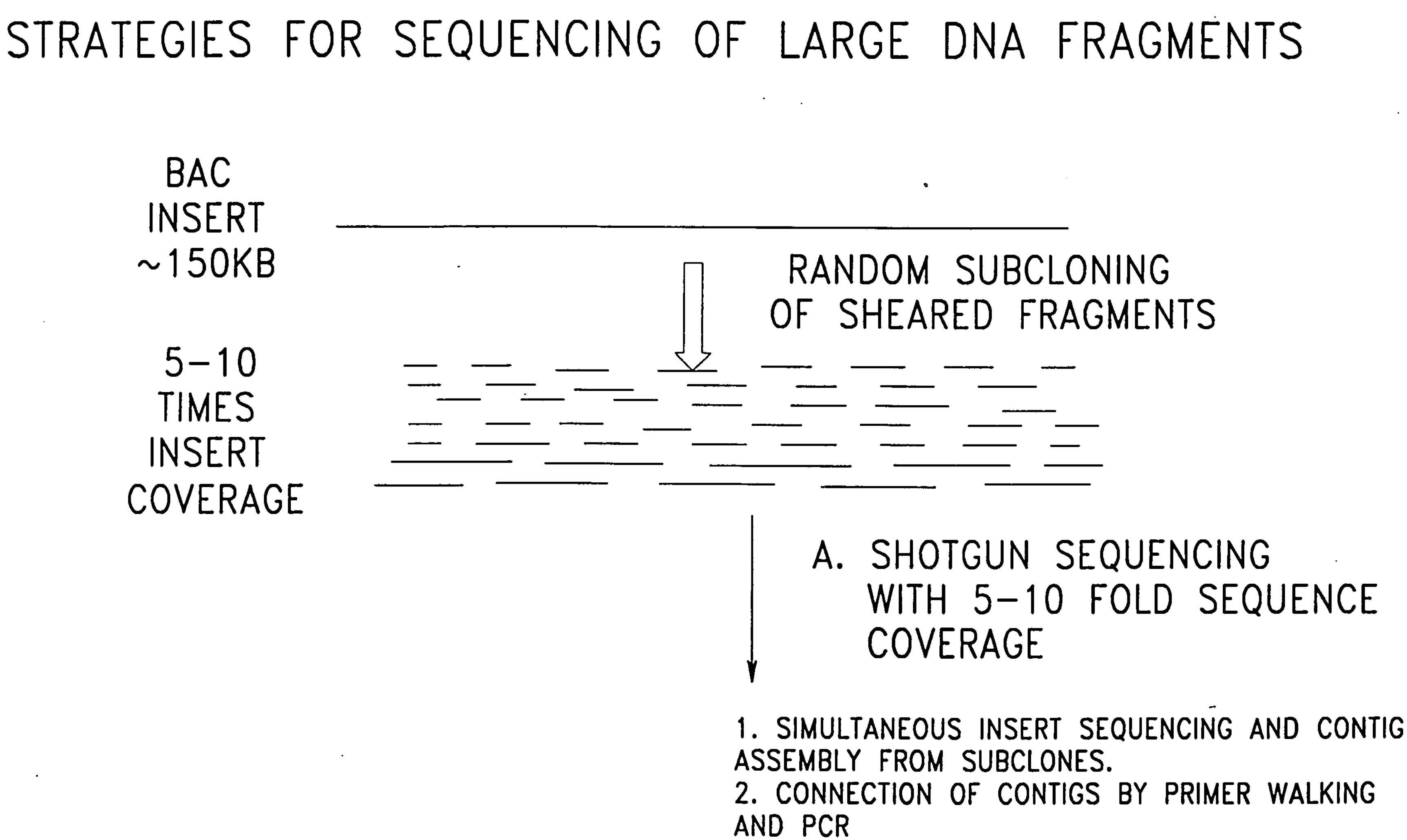

STRATEGIES FOR SEQUENCING OF LARGE DNA FRAGMENTS
BAC
INSERT
~150KB
RANDOM SUBCLONING
OF SHEARED FRAGMENTS
5–10
TIMES
INSERT
COVERAGE
A. SHOTGUN SEQUENCING
WITH 5–10 FOLD SEQUENCE
COVERAGE
1. SIMULTANEOUS INSERT SEQUENCING AND CONTIG
ASSEMBLY FROM SUBCLONES.
2. CONNECTION OF CONTIGS BY PRIMER WALKING
AND PCR

FIG.9A

B. ORDERED SHOTGUN SEQUENCING–OSS

1. SIMULTANEOUS SEQUENCING OF BOTH ENDS OF LIMITED NUMBER OF SUBCLONES(1.5–2 FOLD SEQUENCE COVERAGE).
2. ASSEMBLY OF MINIMAL TILING PATH OF SUBCLONES BY PAIRWISE SEQUENCE OVERLAP.
3. PRIMER WALKING FOR EXTENSIVE SEQUENCING OF MINIMAL TILING PATH SUBCLONES

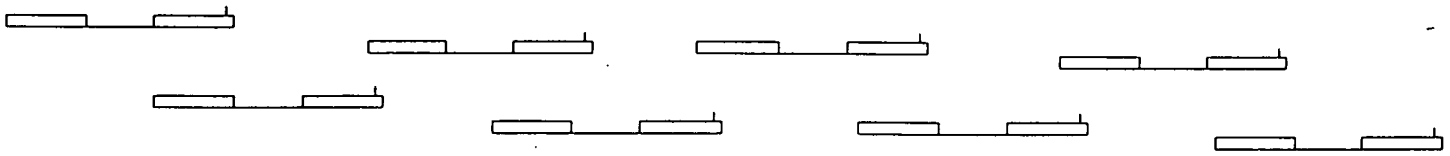


FIG.9B

C. MULTIPLE NUCLEATION POINT WALKING STRATEGY

1. SIMULTANEOUS COMPLETE SEQUENCING OF LIMITED NUMBER OF LARGE INSERT SIZE SUBCLONES WITH PAIRWISE END SEQUENCING FOR THE REST OF THEM RESULTS IN MINIMAL TILING PATH CONTAINING NUCLEATION POINTS OF HIGH QUALITY SEQUENCE.
2. TRANSPOSON MEDIATED SEQUENCING OF MINIMAL TILING PATH.

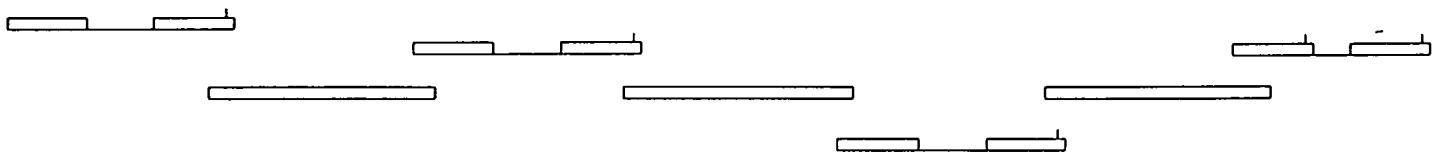


FIG. 9C

	0	50	150	200	250	300	350	400	450	500	550	600	650	700	750	800
PAIRWISE ONLY	29023,2	45244,2	58801,3	68316,9	75206,4	79971,4	83504,1	85720,1	87923,1	88876,5	89630	90447,7	91191,6	91627,5	91925,6	92286,5
MULTIPLE NUCLEATION POINT	0	0	65553,8	83342,9	90466	93252,9	94234,1	94791,3	95127,1	95519,8	95770,1	96043,3	96178,7	96361,7	96443,5	96591,5

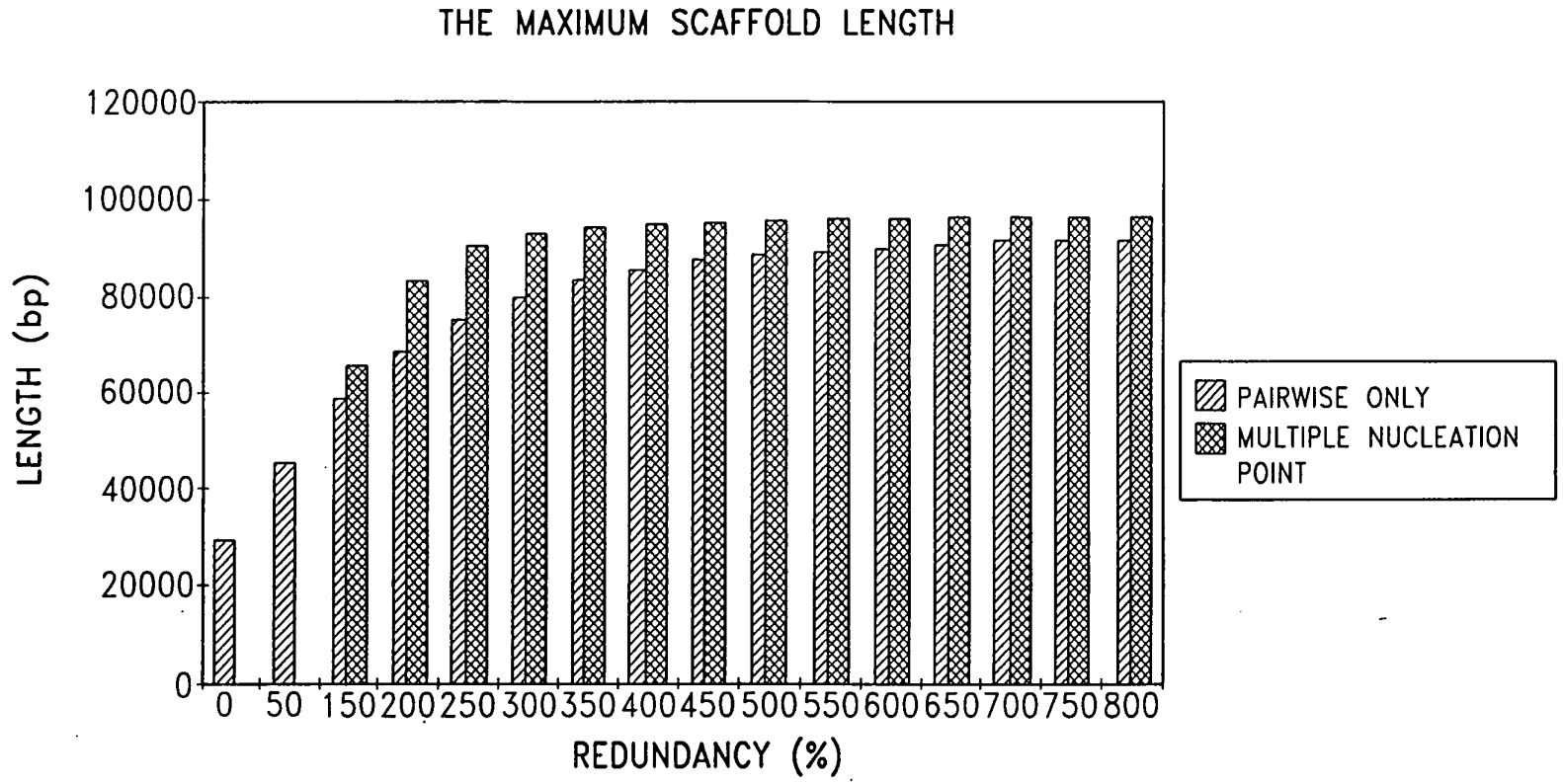


FIG. 10

	50	100	150	200	250	300	350	400	450	500	550	600	650	700	750	800
PAIRWISE ONLY	8,908	16,455	17,729	15,476	12,483	9,701	7,522	6,11	4,974	4,208	3,662	3,24	2,894	2,655	2,467	2,274
MULTIPLE NUCLEATION POINT	0	0	2,5	2,338	2,185	2,046	1,931	1,808	1,676	1,577	1,484	1,43	1,374	1,332	1,302	1,264

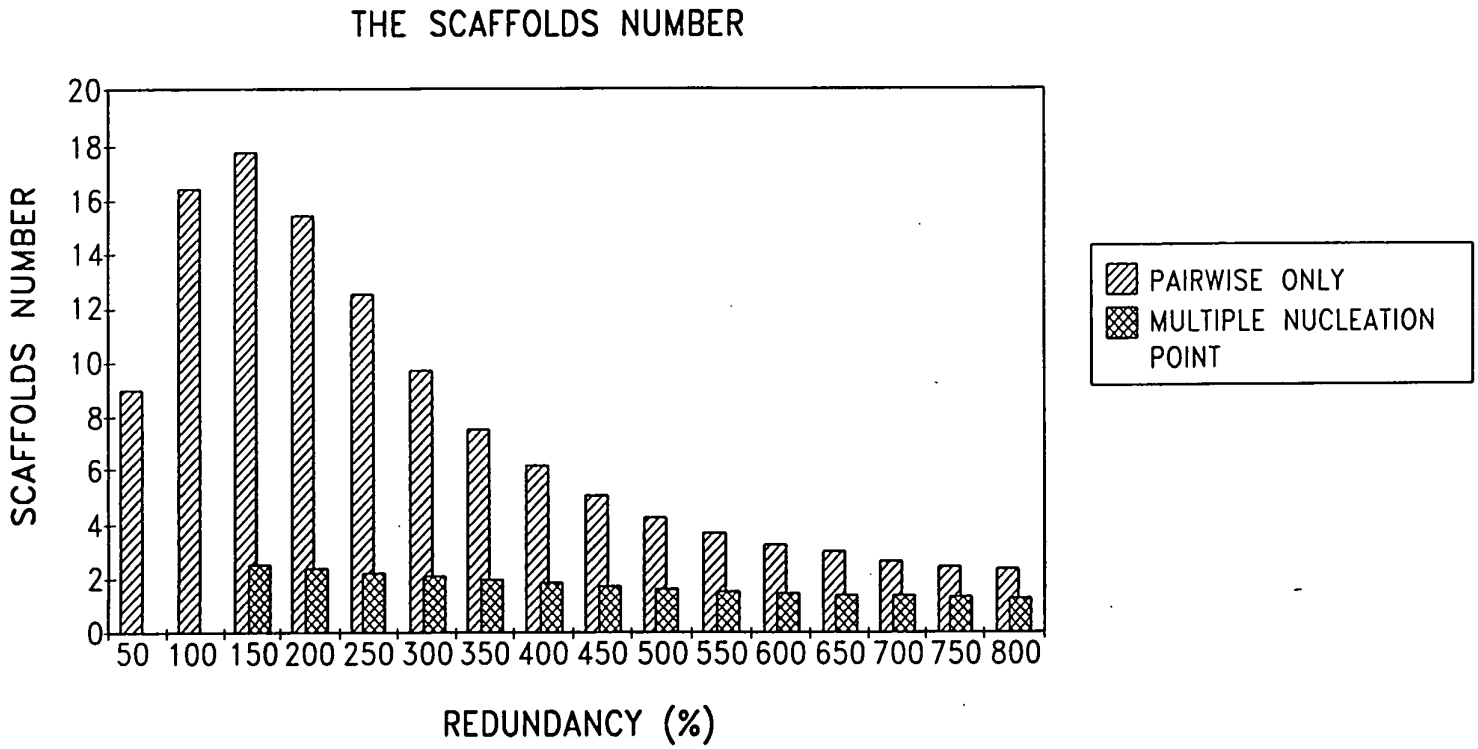


FIG. 11

	50	100	150	200	250	300	350	400	450	500	550	600	650	700	750	800
PAIRWISE ONLY	2,02868	2,64877	3,13585	3,67497	3,77673	3,72902	3,33939	3,00032	2,60141	2,31273	2,12644	1,92935	1,76033	1,64194	1,53327	1,41807
MULTIPLE NUCLEATION	0	0	1,20333	1,59241	1,65915	1,63765	1,53826	1,42167	1,30039	1,16708	1,01968	0,94504	0,83793	0,78088	0,74216	0,66806

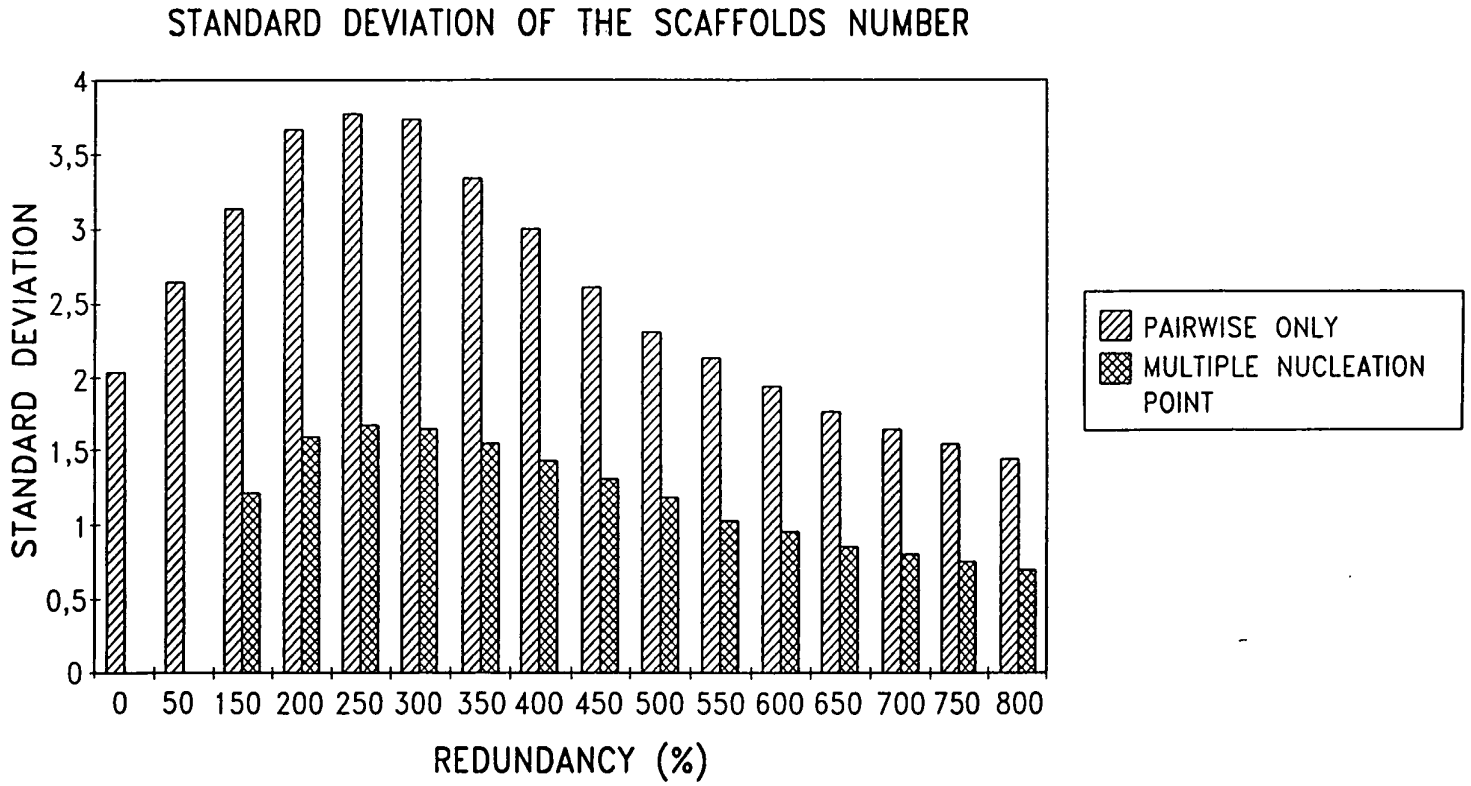


FIG. 12

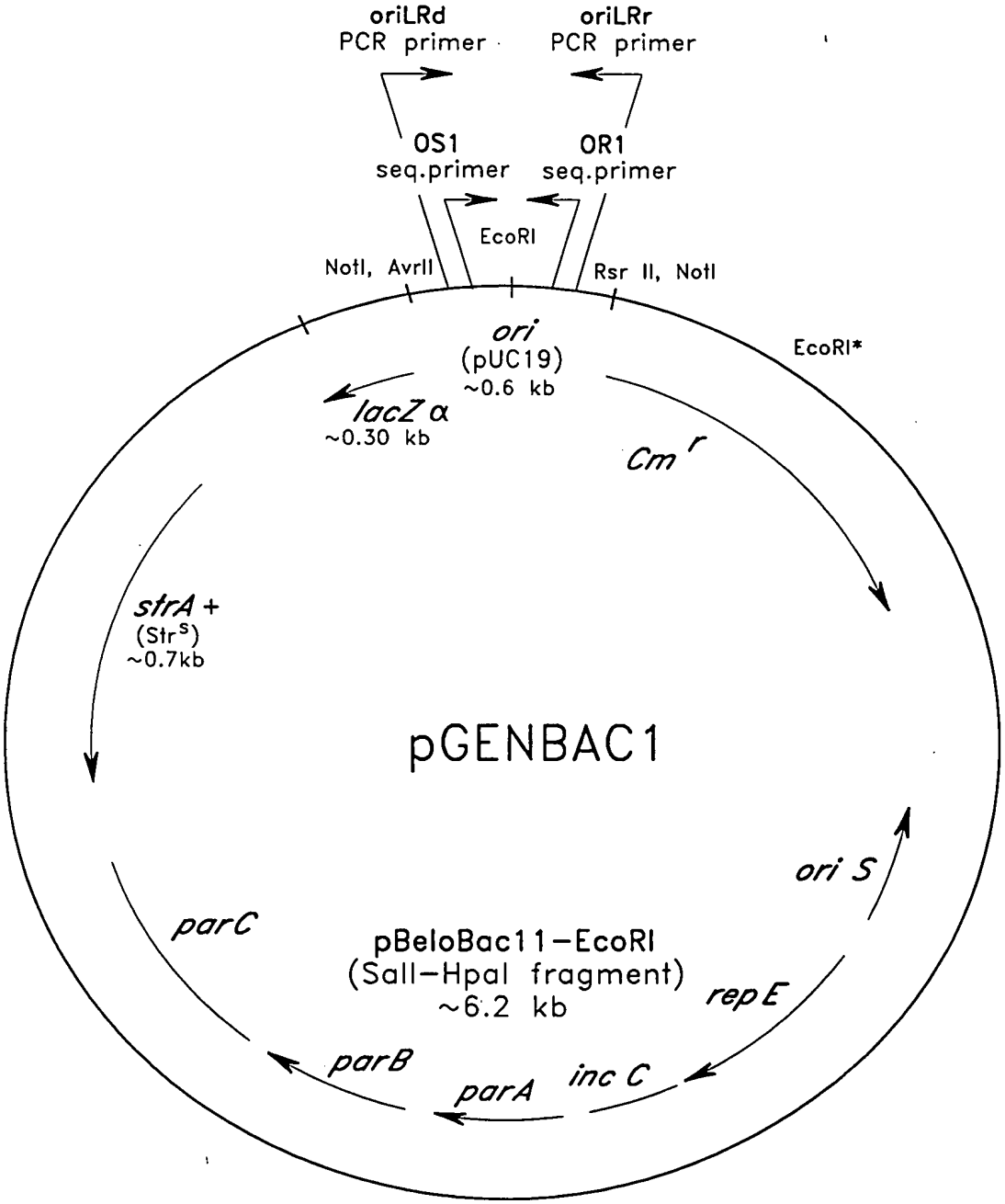

oriLRd
PCR primer
oriLRr
PCR primer
OS1
seq.primer
OR1
seq.primer
EcoRI
NotI, AvrII
Rsr II, NotI
ori
(pUC19)
~0.6 kb
EcoRI*
lacZ α
~0.30 kb
Cm r
strA +
(Str S)
~0.7kb
pGENBAC1
ori S
parC
pBeloBac11-EcoRI
(SalI-HpaI fragment)
~6.2 kb
repE
parB
parA
inc C

FIG. 13

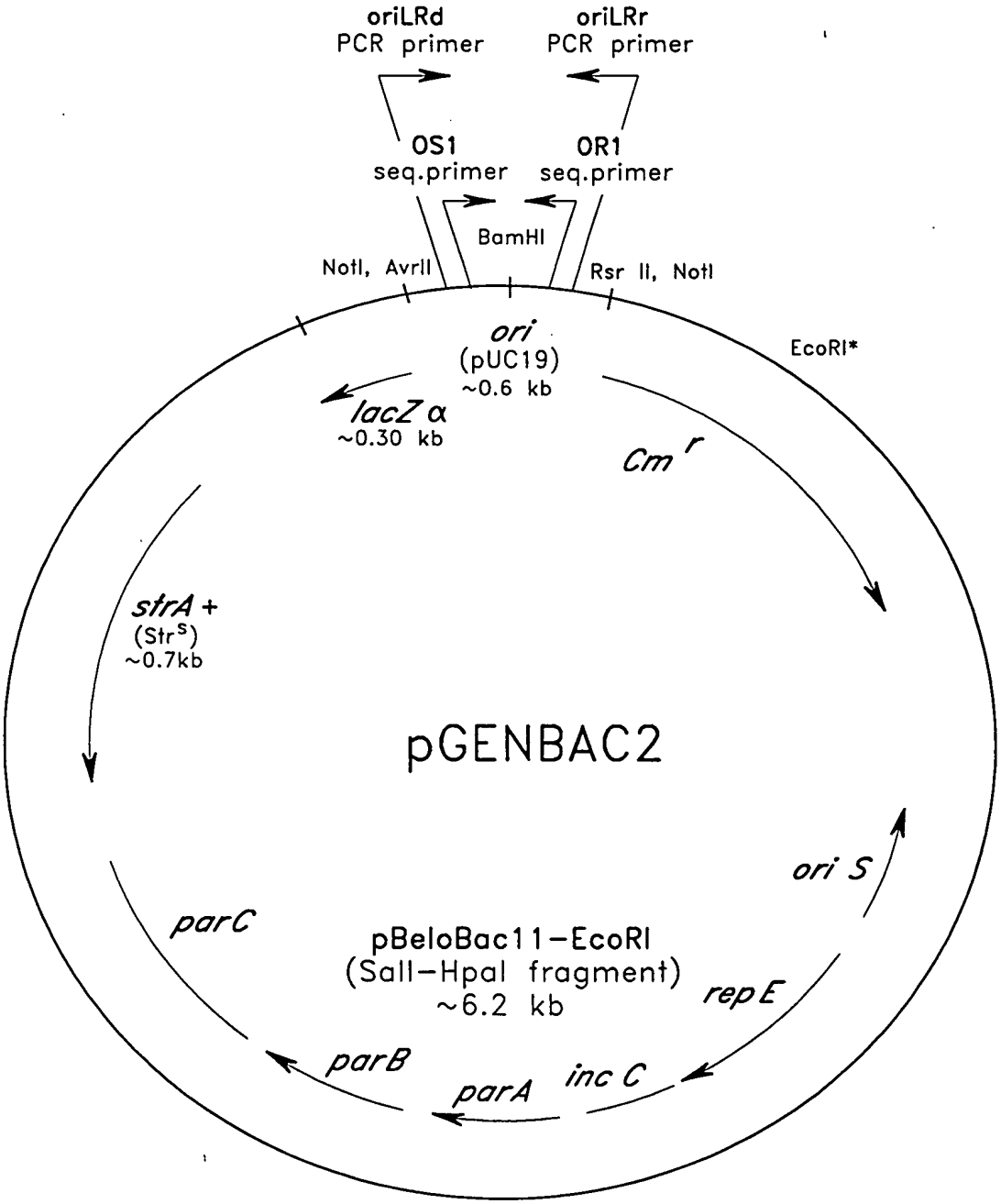

oriLRd
PCR primer
oriLRr
PCR primer
OS1
seq.primer
OR1
seq.primer
BamHI
NotI, AvrII
Rsr II, NotI
ori
(pUC19)
~0.6 kb
EcoRI*
lacZ α
~0.30 kb
Cm r
strA +
(Str S)
~0.7kb
pGENBAC2
ori S
parC
pBeloBac11-EcoRI
(SalI-HpaI fragment)
~6.2 kb
repE
parB
parA
inc C

FIG. 14